



PATENT
28911/34561

MAILED
#117
1/26/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Horton) I hereby certify that this paper is being
Serial No.: 09/027,654) deposited with the United States Postal
Filed: February 23, 1998) Service as first class mail, postage
For: In-Situ Cell Extraction and) prepaid, in an envelope addressed to:
Assay Method) Commissioner for Patents,
Group Art Unit: 1641) Washington, DC 20231 on this date:
Examiner: Gailene R. Gabel) October 22, 2001
) *Jeffery S. Sharp*
) Jeffery S. Sharp
) Registration No. 31,879
) Attorney for Applicants

**DECLARATION OF DR. JEFFREY KENNETH HORTON
UNDER 37 C.F.R. §1.132**

Commissioner for Patents
Washington, DC 20231

Sir:

1. I, Jeffrey Kenneth Horton declare that I am the inventor of the subject matter described and claimed in the above-identified patent application. That I received my Ph.D. in Biochemistry and Cell Biology in 1988 from the Department of Renal Medicine, University of Wales College of Medicine. Since receiving my doctorate, I have been employed by Amersham plc, Cardiff, Wales as a Senior Research Development Scientist working in the area of drug discovery and my Curriculum vitae is attached hereto as Exhibit A.

2. I submit this declaration to address issues raised in the Office Action dated May 22, 2001 in the above-identified application and particularly issues regarding the rejection of the pending claims over the teachings of Lundin *et al.* U.S. 5,558,986 (hereinafter "Lundin") when combined with Cook (Research Focus 1(7): 287-94, 1996) (hereinafter "Cook (1)") and Cook (WO 94/26413) (hereinafter "Cook (2)").

RECEIVED
JAN 23 2002
TECH CENTER 1600/2000

3. The methods claimed in the above-identified patent application are directed to specific binding assays in which the presence of an analyte in a sample is assayed for by steps including reaction of a specific binding partner for the analyte with the analyte to form a specific binding partner-analyte complex and detection of that complex. (One example of a specific binding partner-analyte complex is the complex formed between an antigen and an antibody specific for that antigen.) More particularly, the method of the invention includes the steps of mixing a sample of cells with a cell lysis agent to provide a lysed cellular sample, mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, and performing the specific binding assay in the presence of that sequestrant.

4. The use of a cyclodextrin sequestrant for a cell lysis reagent is not taught in either Cook (1) or Cook (2) for the art of specific binding assays.

5. The use of a cyclodextrin sequestrant for a cell lysis reagent is known in Lundin for conducting various enzyme-mediated reactions. These enzyme-mediated reactions include firefly luciferase assays, polymerase chain reaction (PCR) nucleic acid amplification, and restriction enzyme digestions. Lundin does not teach the use of a cyclodextrin sequestrant for conducting specific binding assays.

6. Assays involving enzyme-mediated reactions, such as those of Lundin, differ from specific binding assays for a specific binding partner in that an enzyme-mediated reaction amplifies the record of the presence of the involved enzyme through the catalysis of the enzymatic reaction to produce a product. Because an enzyme is not consumed in a catalytic reaction the reaction will continue and product will be produced until the reactants are exhausted. Thus, even a minor amount of active enzyme will produce detectable product given sufficient time to complete the reaction. Put another way, even if a large proportion of enzyme is inactivated, any remaining active enzyme will eventually produce detectable product.

7. In contrast to an enzyme-mediated reaction, a specific binding reaction "consumes" the analyte "reactant". The amount of specific binding partner-analyte complex "product" produced will be limited to the amount of biologically active analyte. If a proportion

of the analyte is inactivated by means of a cell lysis agent or otherwise, the remaining portion of active analyte will not take the place of the inactive analyte. Thus, if a sufficiently large proportion of the analyte is inactivated, the remaining small amounts of specific binding partner-analyte complex may be undetectable.

8. For these reasons, the utility of a cyclodextrin sequestrant to provide positive assay results in an enzyme-mediated assay would not lead one of ordinary skill in the art to conclude that similar improvements could be attained in a specific binding assay. Because a specific binding reaction does not include an amplification step in the manner that an enzyme-mediated reaction does, specific binding assays were recognized to be significantly more sensitive to inactivation such as caused by cell lysis reagents. Accordingly, it would not have been clear to one of ordinary skill in the art that incorporation of a cyclodextrin sequestrant would solve the inactivation problem caused by cell lysis reagents.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

DR. JEFFREY KENNETH HORTON

_____, 2001